



Protein Purification
Fong et al.
U.S.S.N. 10/663,483
Docket No. GC775-2
Sheet 1 of 14

1 / 14

General Schematic of Antibody Structure

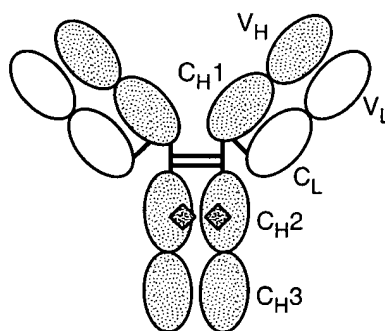
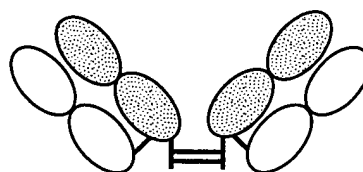
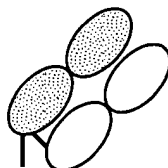


FIG. 1A

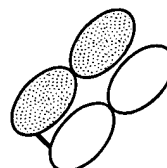
General Schematic of Antibody Fragments



$F(ab')_2$



Fab'



Fab

FIG. 1B

2 / 14

General Schematic of Antibody-Glucoamylase Fusion

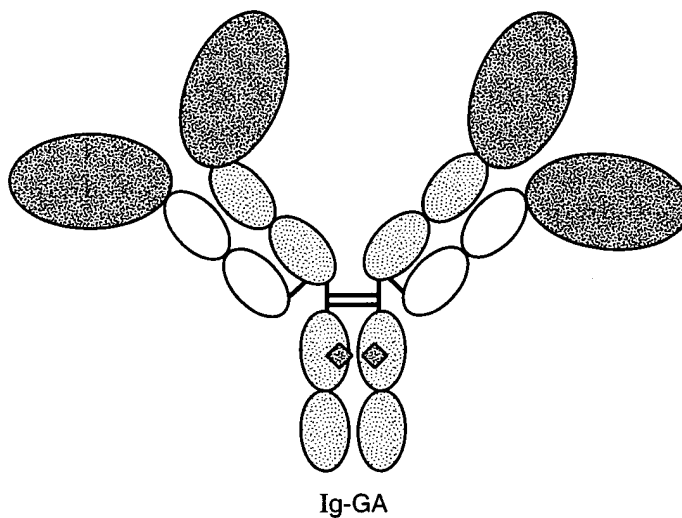
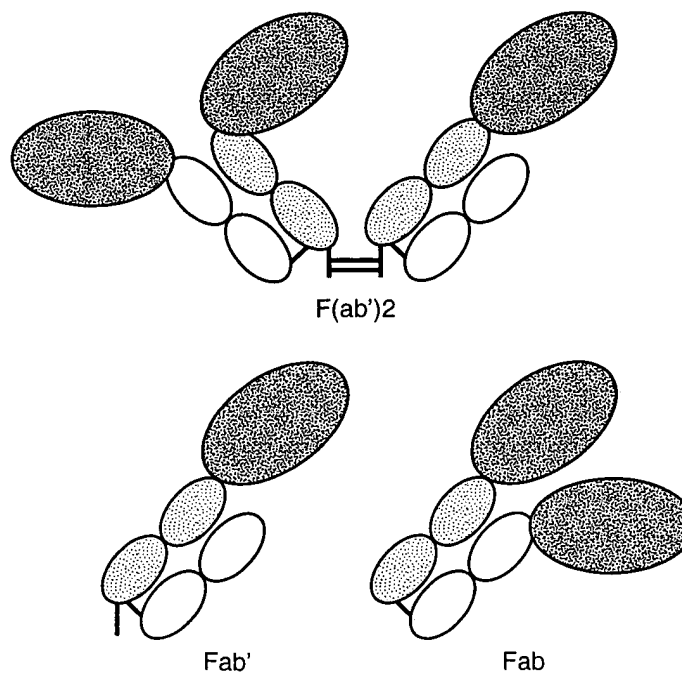


FIG. 1C

General Schematic of Antibody Fragment-Glucoamylase Fusions



Adsorption by Hydrophobic Association

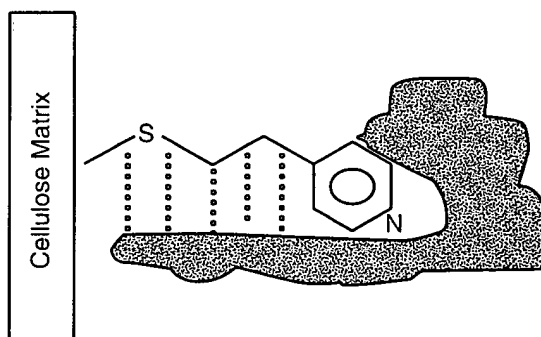


FIG._2A

Desorption by Ionic Repulsion

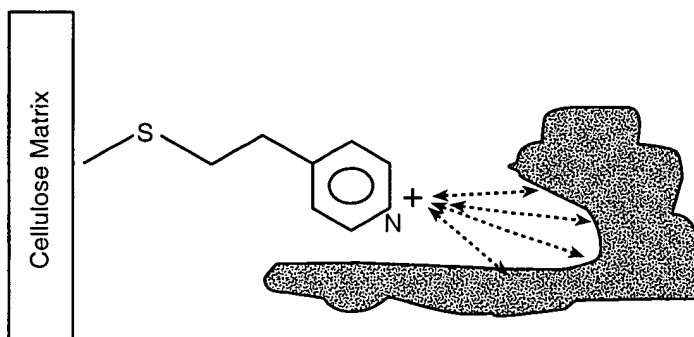


FIG._2B

Sample loaded: 50 mL treated
Fermentation 503 Supernatant
UFC. Treatment includes
neutralization of 500 mL to pH 7.7
with 50 mL 1 M NaOH;
centrifugation 25000xg; and
vacuum filtration through 0.8, 0.45,
and 0.22 μ m filters with 10 g FW12
precoat.

Medium:	Protein A Ceramic
	HyperD F
Column:	HR 16/5, 5.4-mL CV, packed at 10 mL/min, 0.45 MPa
Load Buffer:	25 mM Tris, pH 7.7
Wash Buffer:	25 mM Tris, 500 mM NaCl, pH 7.7
Elution Buffer:	100 mM citrate, pH 2.5

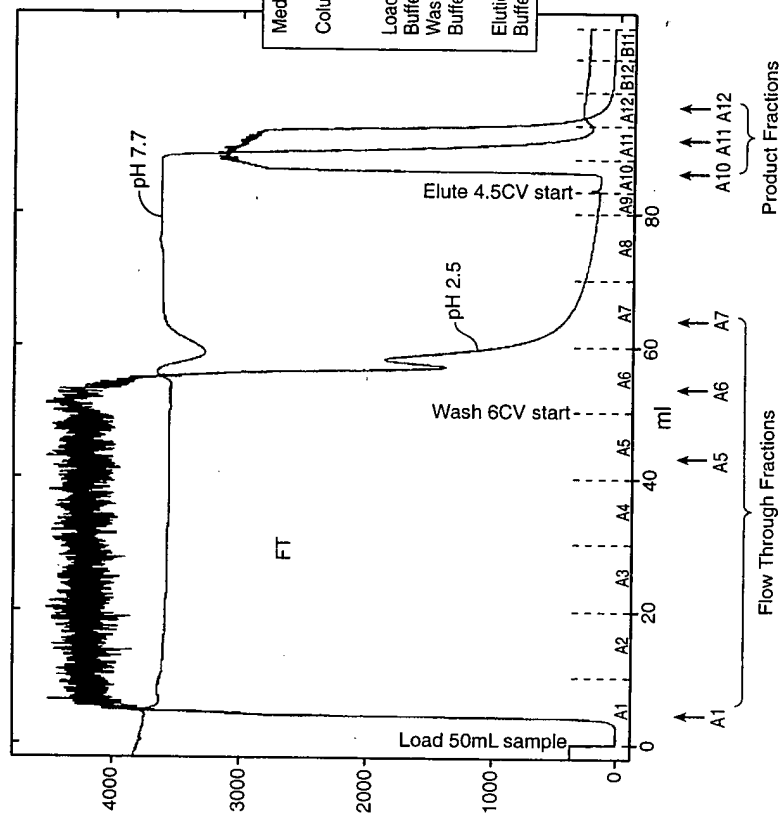


FIG.-3A

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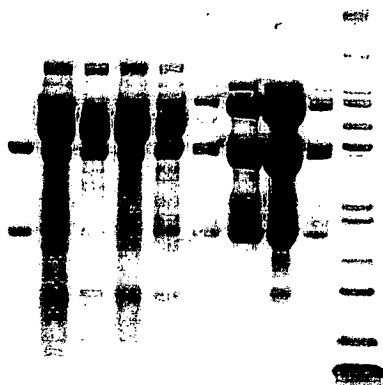
5 / 14

PAC062602

4-12%
 Bis-Tris
 MES
 SDS

Reducing

- Herceptin
 - Feed: 20020503 supernatant
 - A1
 - A5
 - A6
 - A7
 - A10
 - A11
 - A12



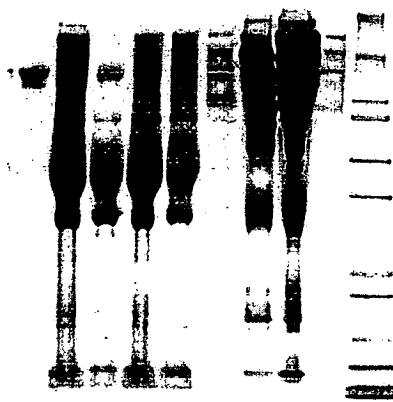
- 200
 - 116
 - 97
 - 66
 - 55
 - 37
 - 31
 - 22
 - 14
 - 6

PAC062602

4-12%
 Bis-Tris
 MOPS
 SDS

Non-reducing

- Herceptin
 - Feed: 20020503 supernatant
 - A1
 - A5
 - A6
 - A7
 - A10
 - A11
 - A12



- 200
 - 116
 - 97
 - 66
 - 55
 - 37
 - 31
 - 22
 - 14

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6 / 14

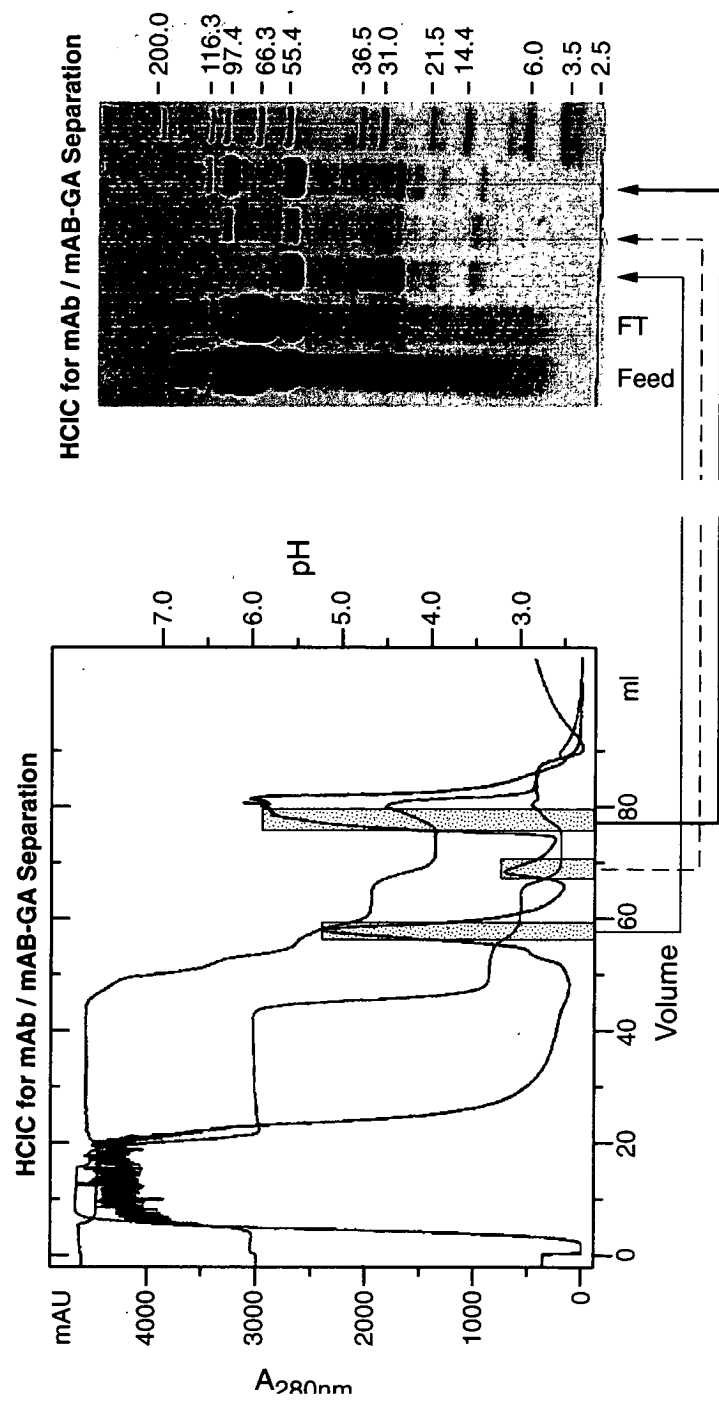


FIG. 4A

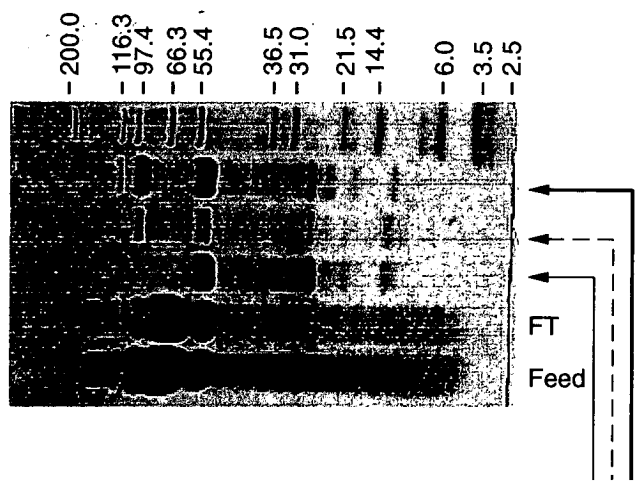


FIG. 4B

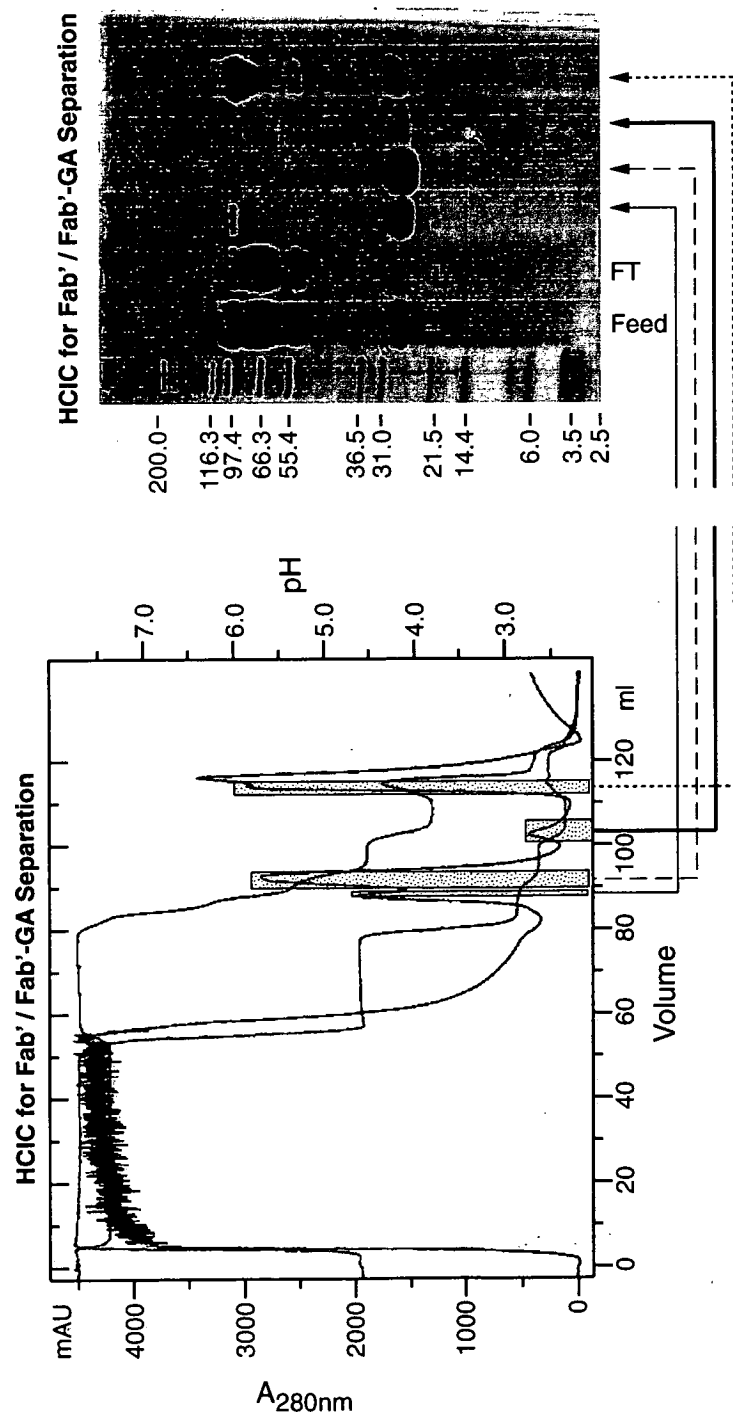


FIG._5A

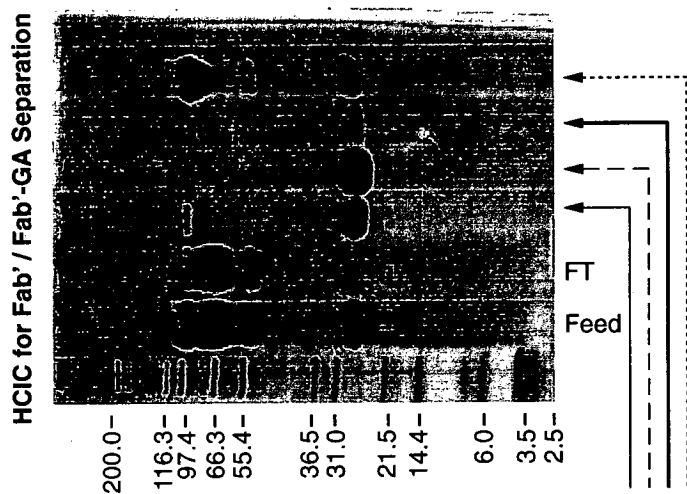


FIG._5B

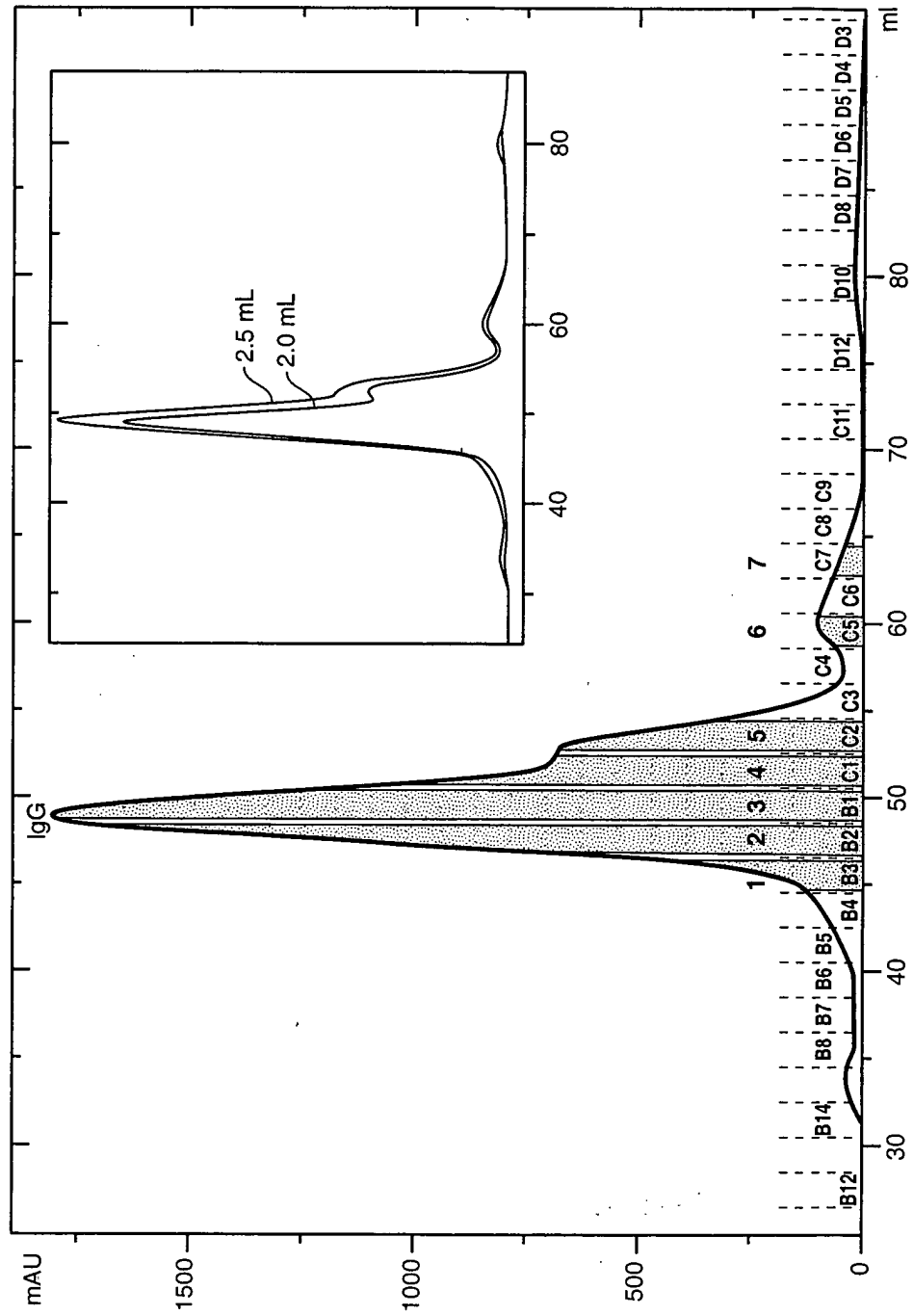
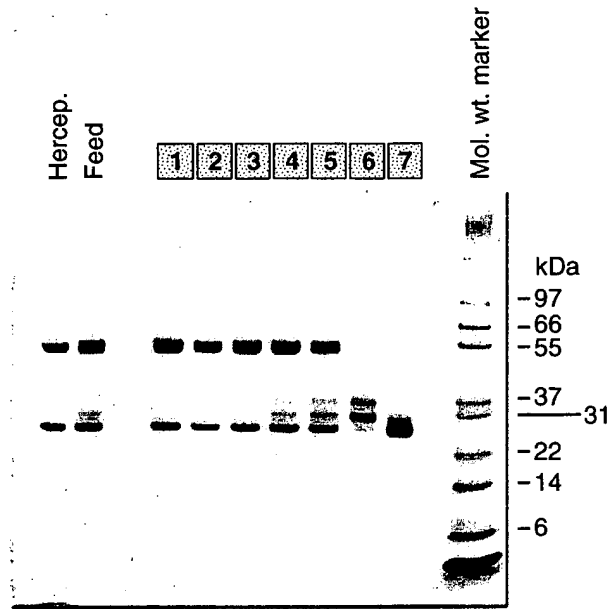


FIG._6A



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FIG._6B

10 / 14

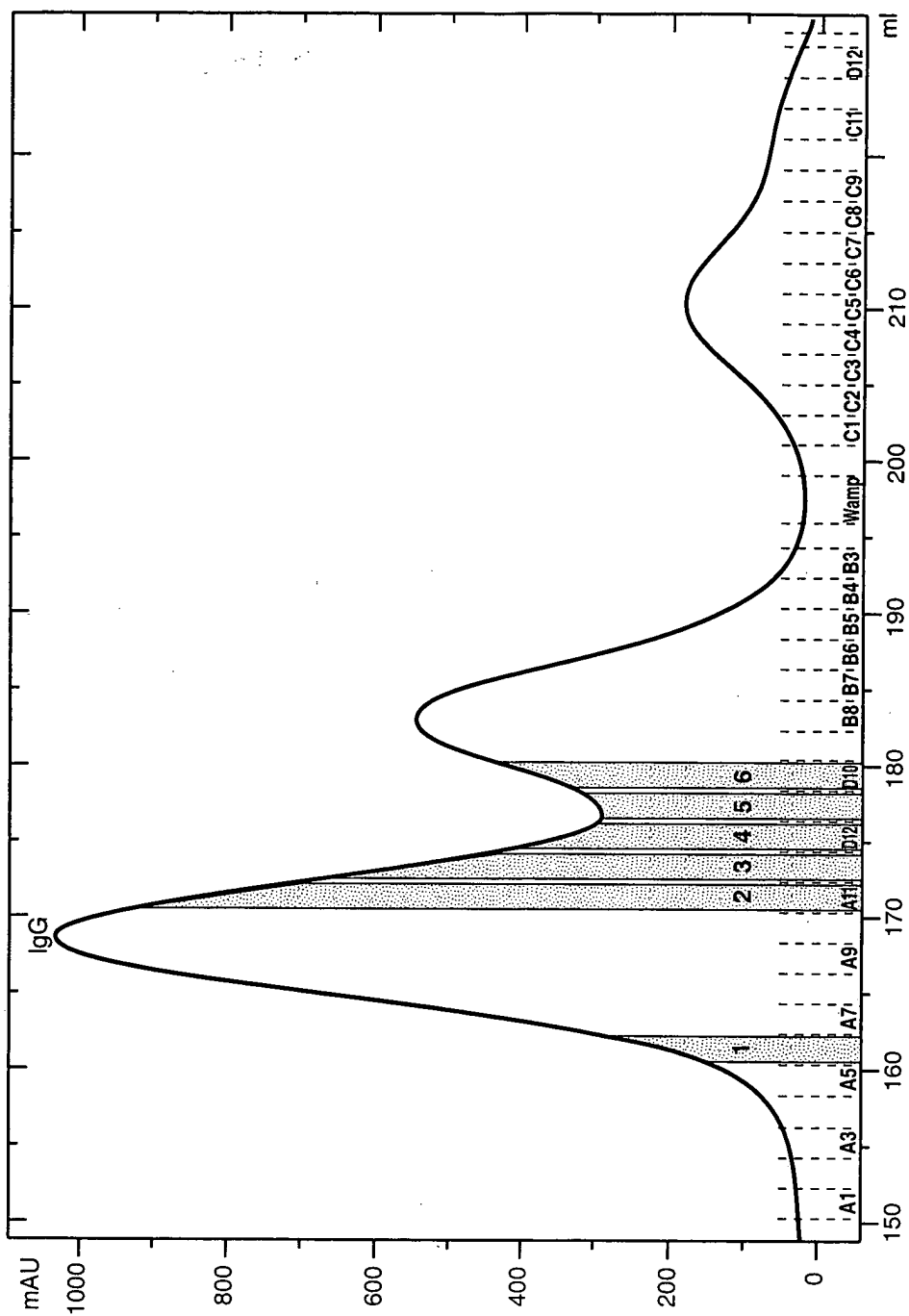


FIG. 7A

11 / 14

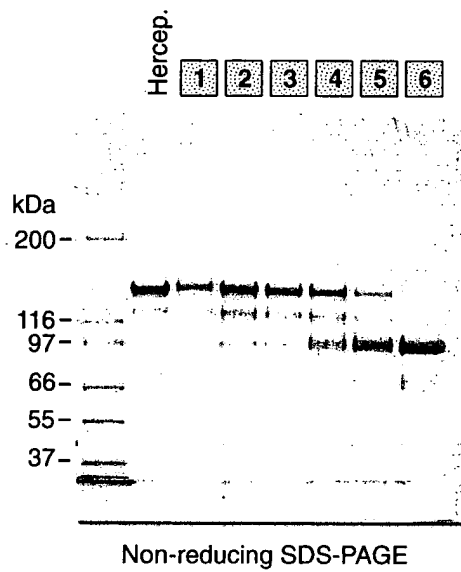
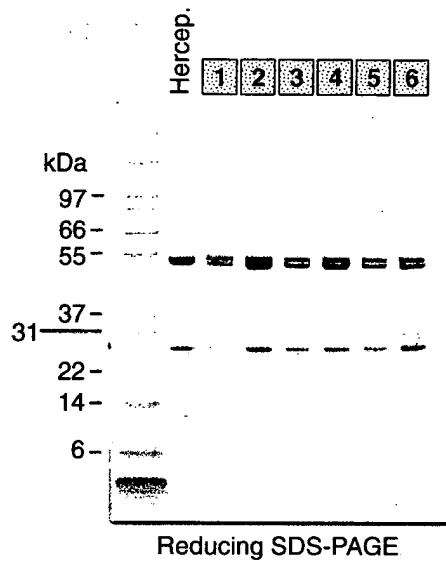


FIG. 7B

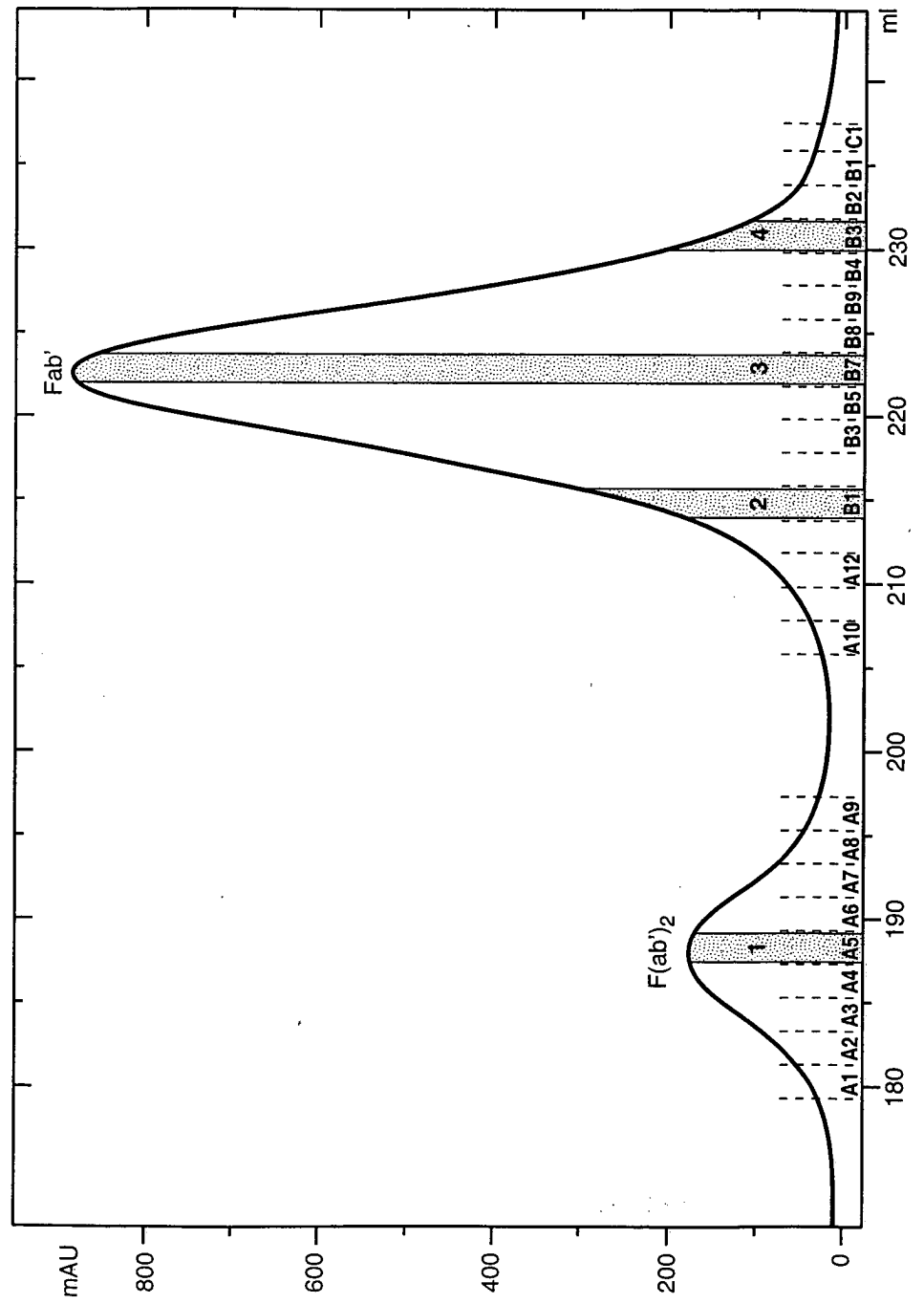


FIG. 8A

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13 / 14

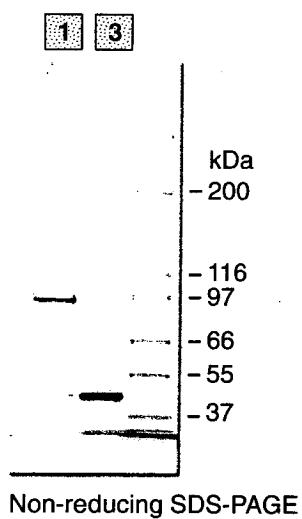
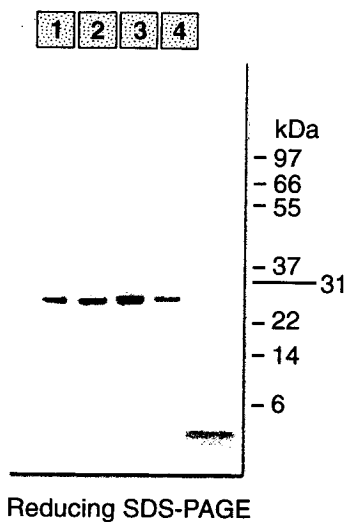
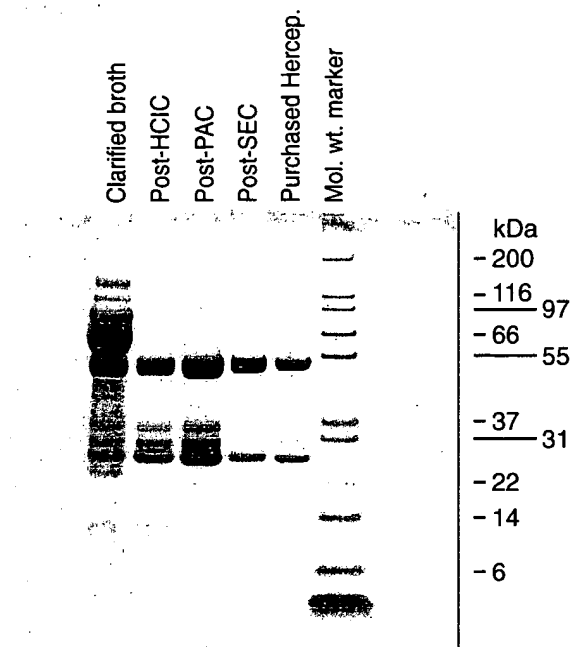


FIG. 8B

14 / 14

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FIG._9